

REMARKS

Status:

Claims 48, 49, 52-59, 62-67 are pending and under consideration, no claims being added, claims 50, 51, 60, and 61 being canceled, and claims 48, 49, 52-54, 56, 58, 62, 63, 65, and 67 being amended herein. Claim 48 is amended to clarify the components of the expression vector used to transform plant cells and to clarify that the transformed plant cell is regenerated into a transgenic plant. Support for the amendment may be found in the specification at least on page 13, lines 14-32 and page 14, lines 20-24. Claim 49 is amended to replace a word which should not be italicized. Claim 52 is amended to further clarify the components of the expression vector of claim 48 used to transform plant cells. Support for this amendment may be found in the specification at least on page 13, lines 5-23. Claims 53 and 54 are amended to further clarify the components of the expression vector of claim 48. Support for the amendments may be found in the specification at least on page 16, lines 1-31. Claim 56 is amended to further clarify the components of the expression vector of claim 52. Support for the amendment may be found in the specification at least on page 18, lines 1-22. Claim 58 is amended to clarify the components of an expression vector used to generate a transgenic plant. Support for the amendment may be found in the specification at least on page 13, lines 5-32 and page 14, lines 20-24. Claims 62 and 63 are amended to further clarify the components of the expression vector of claim 58. Support for the amendments may be found in the specification at least on page 16, lines 1-31. Claim 65 is amended to further clarify the components of the expression vector of claim 58. Support for the amendment may be found in the specification at least on page 18, lines 1-22. Claim 67 is amended to remove the recitation of a drug resistance gene. No new matter is added by these amendments.

Claim 49 was objected to because "*and*" in line 2 should not be italicized. Claims 48-67 were rejected under 35 U.S.C. §112, first paragraph as allegedly containing new matter. Claims 48-67 were rejected under 35 U.S.C. §112, first paragraph as allegedly not enabled. Claims 48, 49, 51-59 and 61-67 were rejected under 35 U.S.C. §112, first

paragraph on the basis that the specification lacked sufficient written description. Claims 48-67 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite. Applicants respectfully traverse these objections and rejections.

Objections:

Claim 49 was objected to because "*and*" in line 2 should not be italicized. Applicants amend claim 49 herein to replace "*and*" with --and--. Applicants respectfully request that the objection be withdrawn.

35 U.S.C. §112, first paragraph, new matter:

Claims 48-67 were rejected under 35 U.S.C. §112, first paragraph as allegedly containing new matter. The Examiner states that the specification and claims do not provide support for the phrases "sarcotoxin 1 family", cecropin family", or "cecropin A". Applicants respectfully disagree. Although Applicants disagree with the Examiner's position, Applicants cancel claims 50, 51, 60 and 61 and amend claims 48, 52-54, 56, 58, 62, 63, and 65 herein to remove these phrases and to limit the claims to sarcotoxin1a. In view of the amendments and remarks, Applicants respectfully request that the §112, first paragraph, new matter rejection be withdrawn.

35 U.S.C. §112, first paragraph, enablement:

Claims 48-67 were rejected under 35 U.S.C. §112, first paragraph as allegedly not enabled. The Examiner acknowledges that the specification is enabling for a method of conferring on a plant, resistance to pathogenic fungi by transforming plant cells with an expression cassette encoding a fusion protein comprising the PR-1a signal peptide, sarcotoxin 1a, the hinge region of the tobacco chitinase and the mature PR-1a operably linked to a dual promoter, and plants so transformed. However, the Examiner states that the specification does not enable the full scope of the claims. Applicants respectfully disagree.

The Examiner states that the specification does not enable a method of conferring resistance to a pathogenic fungi by transformation with an expression cassette encoding a

cecropin or sarcotoxin 1 protein linked to a dual promoter. While Applicants disagree with the Examiner's position, Applicants amend the claims herein to limit the claims to the antibacterial peptide, sarcotoxin 1a. As stated above, the Examiner admits the specification is enabling for a method of conferring on a plant resistance to pathogenic fungi with an expression cassette encoding a sarcotoxin 1a fusion protein.

The Examiner states that the specification fails to provide guidance for expression cassettes where the antibacterial peptide is not part of a fusion protein. Applicants respectfully disagree.

The specification clearly delineates how to construct a vector in which the sarcotoxin 1a gene is fused to a signal sequence at the 5' end and a transcription terminator at the 3' end and the entire fusion gene is operably linked to a stress induced promoter. In Example 1, page 17, line 6 - page 21, line 21 teaches those of skill in the art how to construct the above described fusion protein. This portion of the specification teaches one of skill how to obtain the gene segments encoding the promoter, the signal peptide, sarcotoxin 1a, and a translation termination signal, amplify the gene segments and ligate them together to produce the above described expression cassette. This process is also shown diagrammatically in Figure 6 and the resulting PSS expression cassette is shown schematically in Figure 8. The PSS cassette expresses sarcotoxin 1a fused to DNA encoding a signal sequence but not fused to a sequence encoding a plant gene. Example 1 of the specification further teaches one of skill how to combine the basic expression cassette with other elements to produce an expression cassette encoding a sarcotoxin 1a fusion protein operably linked to dual promoters, shown diagrammatically in Figure 6 and schematically in Figure 8. Example 2, page 22, lines 26-33 teaches one of skill how to put the PSS expression cassette encoding a non-fusion sarcotoxin 1a protein into the pTRA415 plasmid such that the stress inducible promoter is adjacent to the constitutive promoter CaMV 35S. The specification teaches one of skill that the pTRA plasmid is a binary vector containing all of the signals required to mobilize the vector from *Agrobacterium* to a plant (see specification, page 12, line 18 - page 13, line 23. Example 3 teaches one of skill how to transform *Agrobacterium* with a pTRA

plasmid containing the PSS expression cassette. Example 4 teaches one of skill that transformants were obtained expressing the non-fusion (to a plant gene) sarcotoxin 1a protein from the expression cassette PSS. Examples 8, 9, and 10 and data in Tables 1 and 2 and Figure 15 teach one of skill in the art that transgenic tobacco plants expressing a non-fusion (to a plant gene) sarcotoxin 1a protein are resistant to *Rhizoctonia solani*, *Pythium aphanidermatum* and *Phytophthora infestans*. Applicants do state that one embodiment of the present invention is to express sarcotoxin 1a peptide as a fusion protein (see specification, page 16, lines 1-6). However, in view of the above remarks, Applicants submit that they have provided adequate teaching for a method of conferring on a plant resistance to pathogenic fungi by teaching expression cassettes and vectors encoding sarcotoxin 1a fused to a signal peptide but not fused to a second plant protein and provide three working examples of transgenic plants resistant to three different types of pathogenic fungi.

The Examiner also states that expressing cecropins in plants is unpredictable and transforming plants with the gene encoding sarcotoxin 1a is unpredictable. Applicants respectfully disagree.

Although Applicants respectfully disagree with the Examiner's position that expressing cecropins in plants is unpredictable, Applicants amend the claims herein to limit the claims to methods of conferring resistance to pathogenic fungi and transgenic plants resistant to pathogenic fungi by expressing sarcotoxin 1a.

Applicants also respectfully disagree that expressing sarcotoxin 1a in plants is unpredictable. As discussed above, the specification provides working examples of how to make expression constructs expressing sarcotoxin 1a fused to a signal peptide and either not fused to any additional peptides or fused to a second plant peptide. The specification further provides three working examples that both non-fused and fused forms of sarcotoxin 1a were expressed in transgenic plants and the plants were resistant to three different species of pathogenic fungi.

The Examiner relies on Okamoto *et al.*, *Plant Cell Physiol.* 39:57-63 (1998) ("Okamoto") for teaching that the expression of sarcotoxin 1a in plants is unpredictable.

Okamoto teaches five different sarcotoxin 1a constructs (see page 59, Figure 1). The ST10 construct expresses sarcotoxin 1a as a fusion with the PR-1a signal sequence. The STG11 and 12 constructs express sarcotoxin 1a as a fusion with the PR-1a signal sequence and also as a fusion with the bacterial enzyme β -glucuronidase ("GUS") with GUS attached at either the carboxy or amino terminus of Sarcotoxin 1a via a linker consisting of seven serine residues. The STG 13 and 14 constructs express the same sarcotoxin/GUS fusions as STG11 and 12 except the STG 13 and 14 constructs are lacking a signal sequence. Expression of all of the constructs are driven by the CaMV35S promoter.

Okamoto's data actually supports enablement of the above referenced application. Okamoto reports that all of the constructs made are transcribed in transgenic plants with two of the constructs being expressed at slightly higher levels than the other three (see page 59, Figure 3 and text). Although the non-fusion sarcotoxin 1a protein was not observed in western blots (see Figure 4, page 60) Okamoto states that in ST10 transgenics "a considerable level of disease resistance was observe, and its reproducibility and significance was confirmed (in preparation)." (See page 61, column 2 lines 30-33). The ST10 construct of Okamoto is similar to the PSS construct of the above referenced application except that the ST10 construct only contains a single constitutive promoter and not a dual promoter as in the PSS construct. Okamoto notes that expression of sarcotoxin 1a from the ST10 construct did not have any deleterious effect on the phenotype of the transgenics (see page 61, column 1, lines 2-4). These data confirmed other data presented by Okamoto which indicated that exogenously added sarcotoxin 1a had no effect on the growth of tobacco or rice plant cells grown in suspension cultures (see page 60, column 2, lines 6-9). In summary, Okamoto's data support and enable the claimed invention for the following reasons. First, Okamoto's data show that the gene encoding sarcotoxin 1a can be fused to DNA encoding a plant signal sequence and expressed using a constitutively active promoter. Second, Okamoto's data further show that even though sarcotoxin 1a protein is not detected in ST10 transgenic plants, a sufficient amount of protein is made to confer resistance to bacteria in the transgenic

plants. Third, Okamoto's data show that exogenously added sarcotoxin 1a did not affect growth in plant cell suspension cultures.

Okamoto's GUS fusion protein constructs fall outside the scope of the claimed invention and are irrelevant for the purposes of enablement. The Examiner states that transformation with a sarcotoxin 1a gene is unpredictable because Okamoto reported that transgenic plants expressing one of four different sarcotoxin 1a/GUS fusion proteins had abnormal phenotypes and disease resistance was reduced.

These constructs fall outside of the scope of the claimed invention for two reasons. First, the claimed invention is limited to the sarcotoxin 1a gene fused to a **plant gene** and GUS is a bacterial gene. Okamoto speculates that the bacterial gene, when expressed in a plant cell, is inappropriately modified, which could result in improper folding and functioning of both peptides in a fusion protein (see page 61, column 2 and page 62, column 1). Second, the claimed invention is limited to a sarcotoxin 1a/plant gene fusion wherein the two coding sequences are linked via the tobacco chitinase hinge region. The hinge region is a glycine and proline rich region known to connect two distinctly different regions of the tobacco chitinase protein and is expected to prevent steric hindrance of the two separate protein domains (see specification, page 9, lines 25-33). Okamoto linked the sarcotoxin 1a and GUS peptides via seven serine residues. Data was not presented to indicate this linker allows each portion of the fusion protein to function independently. In summary, the Examiner has not presented any evidence to indicate that transformation with the claimed sarcotoxin 1a/plant gene fused via the tobacco chitinase hinge region is unpredictable and the example presented by the Examiner falls outside of the scope of the claims.

The Examiner states that the specification fails to provide guidance for the selection of the promoter induced by stress. The Examiner has failed to provide a reasonable explanation for why any plant promoter induced by stress and any DNA sequence encoding a plant signal peptide cannot be used in the claimed invention. The Examiner is reminded that she has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention (see *In re Wright*, 999 F.2d

1557, 1562 (Fed Cir. 1994)). The Examiner states that undue trial and error experimentation would be required to screen through the myriad of nucleic acids encompassed by the claims and plants transformed therewith, to identify those with increased resistance to pathogenic fungi. However, the Examiner has failed to provide any evidence that plant stress induced promoters and plant signal sequences, other than those disclosed in the specification, would not work. "[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." *In re Colianni* 561 F.2d 220, 224 (CCPA 1977).

Applicants working example is sufficient to enable the claimed genus. Applicants provide a working example wherein the sarcotoxin 1a gene is expressed in an expression cassette wherein the plant promoter induced by stress and DNA encoding the signal sequence are obtained from the PR-1a gene. Several different constructs were generated using these components and all constructs made conferred resistance to pathogenic fungi in transgenic plants carrying them. The Examiner has failed to provide any reasonable explanation as to why other plant promoters induced by stress or other plant signal sequences would not function similarly in the claimed invention. "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.' " *In re Wands*, 858 F.2d 731, 737 (Fed. Cir 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-504 (CCPA 1976)).

Applicants submit that it would be routine experimentation to substitute the PR-1a promoter and signal sequence for another known plant promoter induced by stress and another known DNA sequence encoding a signal peptide. Applicants provide numerous examples for how to construct expression vectors, how to transform plants, and how to determine if the transgenic plants produced using the expression constructs are resistant to pathogenic fungi (see specification examples 1-10). In view of this guidance, it would be routine for one of skill to substitute a different known plant promoter induced by stress

and another DNA sequence encoding a signal peptide with the PR-1a promoter and signal peptide.

Applicants submit that in view of the above remarks, the claims as amended herein are fully enabled and respectfully request that the 112, first paragraph enablement rejection be withdrawn.

35 U.S.C. §112, first paragraph, written description:

Claims 48, 49, 51-59, and 61-67 were rejected under 35 U.S.C. §112, first paragraph on the basis that the specification lacked sufficient written description. The Examiner states that no description is provided in the specification for the structural features of all nucleic acids that encode a cecropin protein or a sarcotoxin 1 protein other than sarcotoxin 1a. Applicants respectfully disagree.

For the reasons discussed below, Applicants disagree with the Examiner's position. However, in view of the Examiner's remarks, Applicants amend the claims herein to limit the claims to a DNA sequence encoding sarcotoxin 1a.

It is well settled that what is conventional or well known to one of ordinary skill in the art need not be disclosed in the specification in detail. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d at 1384. In responses filed December 23, 1999, January 26, 2001, and August 23, 2001, Applicants enclosed references disclosing nucleic acid sequences encoding sarcotoxin 1 or cecropin family members and references disclosing how the structural similarity among the sarcotoxin 1 family members and the cecropin family members relate to functional similarities. These references were available at the time the application was filed. In view of what was commonly known in the art at the time the invention was made, in conjunction with the working examples, methods and nucleic acids disclosed in the specification, Applicants submit that one of skill would have understood the Inventors to be in possession of the claimed invention at the time of filing. In view of the above amendments and remarks, Applicants respectfully request that the 112, first paragraph, written description rejection be withdrawn.

35 U.S.C. §112, second paragraph, indefiniteness:

Claims 48-67 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite. The Examiner states that claim 48 is unclear for several reasons. The Examiner states that lines 2-3 are unclear because it is not known what the phrase "using a DNA . . . Dipteran insect" is intended to modify. The Examiner states that in claim 48, line 4, it is unclear to what the DNA sequence is introduced. The Examiner states that the limitations "the transformed plant" in claim 48, line 5 and "the DNA" in line 7 lack antecedent basis. The Examiner states that in claim 48 it is unclear where the coding sequences are relative to the promoters. The Examiner states that in claim 52 it is unclear if the expression cassette further comprises parts (i) and (ii) or if these parts are intended to further limit the expression cassette of claim 48. The Examiner states that it is unclear in claims 54 and 63 where the signal sequence is located. The Examiner states that it is unclear in claim 58 on what the plant confers resistance to pathogenic fungi. The Examiner states that in claim 67, it is unclear if the drug resistance gene is the same gene as in claim 58, and where it is located in the expression vector.

Applicants amend claims 48, 52, 53, 54, 58, 62, 63 and 67 herein to clarify the subject matter that Applicants regard as their invention. Applicants amend claim 48 herein to address the Examiner's concerns by deleting the phrase "using a DNA . . . Dipteran insect" from lines 2-3, deleting lines 5-9 beginning with "by introducing the DNA" and ending with "from a Diptera insect is in" deleting lines 15-16 and adding -- regenerating the transformed plant cell into a transgenic plant-- in line 17. Applicants amend claim 52 herein by deleting lines 3-5 and reciting that the drug resistance gene is operably linked to the second plant promoter. Claims 53 and 62 are amended herein to recite that nucleotides encoding the tobacco hinge region are located between nucleotides encoding sarcotoxin 1a and a plant gene. Claims 54 and 63 are amended to recite that nucleotide sequences encoding a signal peptide are fused to the nucleotides encoding sarcotoxin 1a. Claim 58 is amended herein to recite that the transgenic plant --is resistant-- to pathogenic fungi rather than conferring resistance to pathogenic fungi. Claim 67 is amended herein to delete the phrase "a drug resistant gene." The locations of

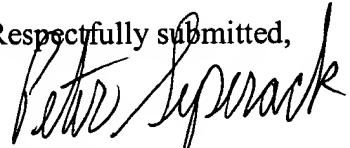
the elements of the expression vectors are exemplified in figure 8. In view of the above amendments, Applicants respectfully request that the 112, second paragraph rejection for indefiniteness be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,


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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1 48. (Amended) A method of conferring resistance to pathogenic fungi on
2 a plant [using a DNA sequence encoding a member of the sarcotoxin 1 family or the
3 cecropin family of antibacterial peptides from a Diptera insect], the method comprising
4 the steps of:

5 transforming a plant cell [by introducing the DNA sequence encoding the
6 member of the sarcotoxin 1 family or the cecropin family; and regenerating the
7 transformed plant cell into a transgenic plant expressing the member of the sarcotoxin 1
8 family or the cecropin family, wherein the DNA encoding the member of the sarcotoxin 1
9 family or the cecropin family from a Diptera insect is in] with an expression vector,
10 wherein said expression vector comprises:

11 [i)] an expression cassette comprising a first plant promoter induced by
12 stress operably linked to a DNA sequence encoding sarcotoxin 1a; and
13 [ii)] a second plant promoter which is constitutively expressed and
14 positioned adjacent to the first plant promoter,

15 [wherein the first plant promoter and the second plant promoter are positioned adjacent to
16 each other,] and

17 regenerating the transformed plant cell into a transgenic plant wherein the
18 transgenic plant has enhanced resistance to pathogenic fungi as compared to a
19 corresponding untransformed plant.

1 49. (Amended) The method according to claim 48, wherein the
2 pathogenic fungi are *Rhizoctonia solani*, *Pythium aphanidermatum*, *[and]* and
3 *Phytophthora infestans*.

1 52. (Amended) The method according to claim 48, wherein said
2 expression vector further comprises[:

3 i) the expression cassette comprising the DNA sequence encoding the
4 member of the sarcotoxin 1 family or the cecropin family operably linked
5 to the first plant promoter; and
6 ii)] a drug resistance gene operably linked to the second plant promoter.

1 53. (Amended) The method according to claim 48, wherein a plant gene
2 is fused to the DNA sequence encoding [the member of the sarcotoxin 1 family or the
3 cecropin family] sarcotoxin 1a [is operably linked to a plant gene] via the hinge region of
4 a tobacco chitinase gene.

1 54. (Amended) The method according to claim 48, wherein a DNA
2 sequence encoding a signal peptide from a plant gene is fused to the DNA sequence
3 encoding [the member of the sarcotoxin 1 family or the cecropin family] sarcotoxin 1a [is
4 operably linked to a signal sequence from a plant gene].

1 56. (Amended) The method according to claim 52, wherein the
2 expression cassette further comprises the terminator of the tobacco PR-1a gene operably
3 linked downstream of the DNA sequence encoding sarcotoxin 1a [the member of the
4 sarcotoxin 1 family or the cecropin family].

1 58. (Amended) A transgenic plant which [confers resistance] is resistant
2 to pathogenic fungi, the plant comprising an expression vector, wherein the expression
3 vector comprises:

4 i) a first [an] expression cassette comprising a DNA sequence encoding
5 sarcotoxin 1a [a member of the sarcotoxin 1 family or the cecropin family
6 of antibacterial peptides from a Diptera insect] operably linked to a
7 promoter induced by stress; and
8 ii) a second expression cassette comprising a drug resistance gene
9 operably linked to a constitutively expressed promoter,
10 wherein the first and second expression cassettes [promoter induced by stress and the
11 constitutively expressed promoter] are positioned adjacent to each other, and wherein the

12 transgenic plant has enhanced resistance to pathogenic fungi as compared to a
13 corresponding untransformed plant.

1 62. (Amended) The plant according to claim 58, wherein a plant gene is
2 fused to the DNA sequence encoding sarcotoxin 1a [the member of the sarcotoxin 1
3 family or the cecropin family is operably linked to a plant gene] via the hinge region of a
4 tobacco chitinase gene.

1 63. (Amended) The plant according to claim 58, wherein a DNA
2 sequence encoding a signal peptide from a plant gene is fused to the DNA sequence
3 encoding sarcotoxin 1a in the first expression cassette [member of the sarcotoxin 1 family
4 or the cecropin family is operably linked to a signal sequence from a plant gene].

1 65. (Amended) The plant according to claim 58, wherein the first
2 expression cassette further comprises the terminator of the tobacco PR-1a gene operably
3 linked downstream of the DNA sequence encoding sarcotoxin 1a [the member of the
4 sarcotoxin 1 family or the cecropin family].

1 67. (Amended) The plant according to claim 58, wherein the expression
2 vector further comprises a T-DNA region [and a drug resistance gene].